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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No.	MSI 100
First Inventor or Application Identifier	Frank B. Dean
Title	METHOD FOR REDUCING ARTIFACTS IN NUCLEIC ACID AMPLIFICATION
Express Mail Label No.	EL 320 550 963 US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: **Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231**

1. [X] * Fee Transmittal Form *(e.g., PTO/SB/17)*
(Submit an original and a duplicate for fee processing)

2. [X] Specification [*Total Pages* 36]
(preferred arrangement set forth below)
- Descriptive title of the Invention
- Cross References to Related Applications
- Statement Regarding Fed sponsored R & D
- Reference to Microfiche Appendix
- Background of the Invention
- Brief Summary of the Invention
- Brief Description of the Drawings *(if filed)*
- Detailed Description
- Claim(s)
- Abstract of the Disclosure

3. [X] Drawing(s) *(35 U.S.C. 113)* [*Total Sheets* 7]

4. Oath or Declaration [*Total Pages* 3]
   a. [X] Unexecuted
   b. [ ] Copy from a prior application (37 C.F.R. § 1.63(d)) *(for continuation/divisional with Box 16 completed)*
      i. [ ] DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1.63(d)(2) and 1.33(b).

** NOTE FOR ITEMS 1 & 13: IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 C.F.R. § 1.27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C.F.R. § 1.28).*

5. [ ] Microfiche Computer Program *(Appendix)*

6. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. [ ] Computer Readable Copy
   b. [ ] Paper Copy (identical to computer copy)
   c. [ ] Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

7. [ ] Assignment Papers (cover sheet & document(s))
8. [ ] 37 C.F.R.§3.73(b) Statement *(when there is an assignee)* [ ] Power of Attorney
9. [ ] English Translation Document *(if applicable)*
10. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
11. [ ] Preliminary Amendment
12. [ ] Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
13. [ ] * Small Entity Statement(s) *(PTO/SB/09-12)* [ ] Statement filed in prior application, Status still proper and desired
14. [ ] Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
15. [ ] Other: .....

16. **If a CONTINUING APPLICATION,** *check appropriate box, and supply the requisite information below and in a preliminary amendment:*

[ ] Continuation [ ] Divisional [ ] Continuation-in-part (CIP) of prior application No: _____/_____

Prior application information: *Examiner* _____ *Group / Art Unit:* _____

For CONTINUATION or DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 4b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

17. CORRESPONDENCE ADDRESS

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Frank B. Dean and Fawad Faruqi	
Serial No:	Not Yet Assigned	Express Mail Label No. EL 320 550 963 US
Filed:	February 25, 2000	Date of Deposit February 25, 2000

For: *METHOD FOR REDUCING ARTIFACTS IN NUCLEIC ACID AMPLIFICATION*

Assistant Commissioner for Patents
Washington, D.C. 20231

EXPRESS MAIL TRANSMITTAL LETTER
FOR PATENT APPLICATION AND CERTIFICATE OF MAILING

Sir:

Pursuant to 35 U.S.C. § 21(a) as amended by Public Law 97-247 and 37 C.F.R. § 1.10, Frank B. Dean and Fawad Faruqi enclose for filing the attached patent application which is entitled "Method for Reducing Artifacts in Nucleic Acid Amplification". The application includes 1 page of Abstract, 25 pages of Specification, 10 pages of Claims, 7 sheets of Drawings, and an Unexecuted Declaration. A Verified Statement Claiming Small Entity Status will be submitted shortly. A check in the amount of $927.00 to cover one half of the filing fee is enclosed.

The Commissioner is hereby authorized to charge our Deposit Order Account No. 01-2507 in the amount of $927.00, which represents the difference between the filing fee for a large entity and a small entity.

MSI 100
15122/2

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This application is being filed on February 25, 2000 by mailing the application to the Assistant Commissioner for Patents, Washington, D.C. 20231 via the United States Postal Service "Express Mail Post Office to Addressee" Service under 37 C.F.R. § 1.10. The Express Mail Label No. EL 320 550 963 US appears in the heading of this paper which is attached to the application papers pursuant to 37 C.F.R. § 1.10(b).

The Commissioner is hereby authorized to charge any fees that may be required, or credit any overpayment to Deposit Order Account No. 01-2507. To facilitate this process, applicant has enclosed a duplicate of this letter.

All correspondence concerning this application should be mailed to:

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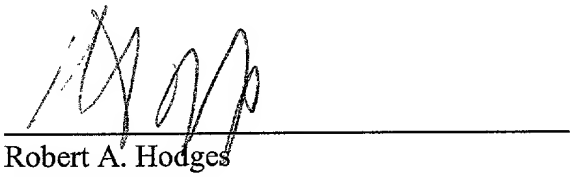
Respectfully submitted,



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Title: METHOD FOR REDUCING ARTIFACTS IN NUCLEIC ACID AMPLIFICATION
By: Frank B. Dean and Fawad Faruqi
Filed: February 25, 2000
Express Mail Transmittal Letter for Patent Application
Express Mail No.: EL 320 550 963 US

CERTIFICATE OF MAILING UNDER 37 CFR § 1.10

I hereby certify that this Express Mail Transmittal Letter for Patent Application and any documents referred to as attached therein are being deposited with the United States Postal Service on this date, February 25, 2000 in an envelope as "Express Mail Post Office to Addressee" service under 37 CFR § 1.10, Mailing Label Number EL 320 550 963 US, addressed to Box Patent Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

Teresa R. Spratt

Date: February 25, 2000

MSI 100
15122/2

APPLICATION

FOR

UNITED STATES LETTERS PATENT

BY

FRANK B. DEAN

AND

FAWAD FARUQI

FOR

METHOD FOR REDUCING ARTIFACTS IN NUCLEIC ACID AMPLIFICATION

METHOD FOR REDUCING ARTIFACTS IN NUCLEIC ACID AMPLIFICATION

BACKGROUND OF THE INVENTION

The present invention is in the field of nucleic acid amplification, and specifically in the area of reducing amplification artifacts in nucleic acid amplification reactions.

Numerous nucleic acid amplification techniques have been devised, including strand displacement cascade amplification (SDCA)(referred to herein as exponential rolling circle amplification (ERCA)) and rolling circle amplification (RCA)(U.S. Patent No. 5,854,033; PCT Application No. WO 97/19193; Lizardi *et al.*, *Nature Genetics* 19(3):225-232 (1998)); multiple displacement amplification (MDA)(PCT Application WO 99/18241); strand displacement amplification (SDA)(Walker *et al.*, Nucleic Acids Research 20:1691-1696 (1992), Walker *et al.*, Proc. Natl. Acad. Sci. USA 89:392-396 (1992)); polymerase chain reaction (PCR) and other exponential amplification techniques involving thermal cycling, self-sustained sequence replication (3SR), nucleic acid sequence based amplification (NASBA), and amplification with Qβ replicase (Birkenmeyer and Mushahwar, *J. Virological Methods* 35:117-126 (1991); Landegren, *Trends Genetics* 9:199-202 (1993)); and various linear amplification techniques involving thermal cycling such as cycle sequencing (Craxton *et al.*, *Methods Companion Methods in Enzymology* 3:20-26 (1991)).

Artifacts--that is, unwanted, unexpected, or non-specific nucleic acid molecules--have been observed in almost all nucleic acid amplification reactions. For example, Stump *et al.*, *Nucleic Acids Research* 27:4642-4648 (1999), describes nucleic acid artifacts resulting from an illegitimate PCR process during cycle sequencing. Stump *et al.* suggests a way to avoid such artifacts by using certain primers that cannot be fully replicated. Watson, *Amplifications*, 5-6 (1989), and Ferrie *et al.*, *Am. J. Hum. Genet.* 51:251-262 (1992), describe formation of primer-dimer artifacts during PCR. Brownie *et al.*, *Nucleic Acids Research* 25:3235-3241 (1997), suggests a way to avoid

primer dimer formation during PCR by adding tails at the 5' end of the PCR primers that results in formation of a non-replicable structure if primer-dimer formation is initiated. Other forms of artifacts can occur in other types of nucleic acid amplification techniques.

Therefore, it is an object of the present invention to provide a method of reducing, preventing, or eliminating artifacts in nucleic acid amplification reactions.

It is another object of the present invention to provide oligonucleotides that, when used in a nucleic acid amplification reaction, can reduce, prevent, or eliminate artifacts in the nucleic acid amplification reaction.

It is another object of the present invention to provide kits for nucleic acid amplification that can reduce, prevent, or eliminate artifacts in the nucleic acid amplification reaction.

BRIEF SUMMARY OF THE INVENTION

Disclosed are compositions and methods useful for reducing the formation of artifacts during nucleic acid amplification reactions. The method uses special oligonucleotides, referred to herein as template-deficient oligonucleotides, that cannot serve as a template for nucleic acid synthesis over part of their length. This prevents the oligonucleotides from serving as effective templates in the formation of artifacts. The disclosed method involves using a template-deficient oligonucleotide as at least one of the oligonucleotides (preferably a primer) in a nucleic acid amplification reaction, where the template-deficient oligonucleotide comprises one or more template-deficient nucleotides, preferably at or near the 5' end of the template-deficient oligonucleotide. The disclosed method is useful for reducing artifacts in any nucleic acid amplification reaction involving oligonucleotides. In a preferred form of the method the nucleic acid amplification reaction does not involve thermal cycling. The disclosed method is effective at reducing non-cycle oligonucleotide-based artifacts. Also disclosed are kits useful for reducing artifacts in nucleic acid amplification reactions. The disclosed kits include a

template-deficient oligonucleotide, wherein the template-deficient oligonucleotide comprises one or more template-deficient nucleotides, and a nucleic acid polymerase.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram of the proposed "templating" process leading to formation of artifacts.

Figure 2 is a diagram of an oligonucleotide with an abasic nucleotide at the 5' end showing that it cannot serve as a template for synthesis of a complete complementary strand.

Figure 3 is a diagram of an oligonucleotide with two abasic nucleotides at the 5' end showing that it cannot serve as a template for synthesis of a complete complementary strand.

Figure 4 is a diagram showing how primer dimers could be formed during PCR through non-template (that is, overhanging) addition of an adenosine at the 3' end of the synthesized strand.

Figure 5 is a diagram of various structures of examples of the disclosed template-deficient oligonucleotides (oligonucleotides 2-15). The region of each oligonucleotide that is template-deficient, template-capable, primer-deficient, and primer-capable is indicated. For comparison, oligonucleotide 1 is a normal oligonucleotide (that is, composed entirely of template- and primer-capable nucleotides).

Figure 6 is a graph of amount of nucleotide incorporated (in pmol/4 l) versus time (in minutes) during exponential rolling circle amplification reactions using various combinations of control primers (P1 and P2) and abasic primers (P1(aba) and P2(aba)). The abasic primers had two abasic nucleotides at the 5' end. The primers used (and whether a template circle was present) are indicated by annotations to the right of the graph next to the last data point in each curve. The five annotations at the bottom (P1(aba) + P2(aba) - circle, P1 alone, P2 alone, P1(aba) alone, and P2(aba) alone) represent reactions having identical curves showing no nucleotide incorporation. The reaction number, as described

in Example 1, represented by each curve is indicated in parentheses next to the annotations.

Figure 7 is a graph of amount of nucleotide incorporated (in pmol/4 l) versus time (in hours) during exponential rolling circle amplification reactions using various combinations of control primers (P1 and P2) and template-deficient primers (P1aba, P2aba, P1C, and P4C). The P1aba and P2aba primers had two abasic nucleotides at the 5' end. The P1C and P4C primers were chimeric, having 6 deoxyribonucleotides at the 3' end and 18 ribonucleotides at the 5' end. The primers used (and whether a template circle was present) are indicated by annotations to the right of the graph next to the last data point in each curve. The five annotations at the bottom (P1aba + P2aba, P1C + P4C, P1 + P4C, P1aba + P4C, and P1C + P2aba) represent reactions having identical curves showing no nucleotide incorporation.

DETAILED DESCRIPTION OF THE INVENTION

Nucleic acid amplification techniques using primers are often compromised by the formation of nucleic acid artifacts that are not the product of legitimate amplification. For example, exponential rolling circle amplification (ERCA), an isothermal amplification reaction using two primers complementary to opposite strands of a nucleic acid molecule to be amplified (see strand displacement cascade amplification in PCT Application No. WO 97/19193), sometimes results in formation of oligonucleotide-based artifacts. These artifacts, referred to herein as non-cycle oligonucleotide-based artifacts, are different in structure from primer dimer artifacts that can occur in amplification reactions involving thermal cycling and result from a different mechanism.

For example, artifactual DNA products synthesized by Bst polymerase during ERCA consist of DNA molecules that are long, many kilobase in length, yet occur in the absence of template, or any DNA of like size that could act as a template to yield a product of such a size. The artifact DNA synthesis can occur efficiently in the presence of just two short oligonucleotide primers and Bst

alone. The artifactual DNA appears as a collection of products varying greatly in size that appears as a broad smear, or ladder of closely spaced bands, when analyzed by gel electrophoresis.

It has been discovered that non-cycle oligonucleotide-based artifacts could be reduced by using oligonucleotides that could not be replicated to their full length, thereby preventing synthesis of a duplex, blunt-ended structure. Such oligonucleotides are referred to herein as template-deficient oligonucleotides. Such oligonucleotides would be poorly extended out to the ends and would only poorly be able to extend across the nick that is created by the insertion of an interloping oligonucleotide (see Figure 2).

Disclosed are compositions and methods useful for reducing the formation of primer artifacts during nucleic acid amplification reactions. The method uses special oligonucleotides, referred to herein as template-deficient oligonucleotides, that cannot serve as a template for nucleic acid synthesis over part of their length. This prevents the oligonucleotides from serving as effective templates in the formation of artifacts during a nucleic acid amplification reaction. It is preferred that the template-deficient oligonucleotide be a primer, that all of the primers used in the nucleic acid amplification reaction are template-deficient, and/or that all of the oligonucleotides used in the nucleic acid amplification reaction are template-deficient.

In one form, the disclosed method involves using a template-deficient oligonucleotide where the template-deficient oligonucleotide includes one or more template-deficient nucleotides, where the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end of the template-deficient oligonucleotide is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction. Template-deficient oligonucleotides of this form are referred to herein as 3' open oligonucleotides. Template-deficient oligonucleotides referred to herein as 3' closed oligonucleotides are template-

deficient oligonucleotides where the template-deficient oligonucleotide includes one or more template-deficient nucleotides, where the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end of the template-deficient oligonucleotide is insufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction. Put another way, placing a template-deficient nucleotide at or near the 3' end of an oligonucleotide results in a 3' closed oligonucleotide.

In another form, the disclosed method involves using a template-deficient oligonucleotide where the template-deficient oligonucleotide includes one or more template-deficient nucleotides.

Numerous configurations of template-deficient nucleotides can be used in the disclosed template-deficient oligonucleotides. For example, when the template-deficient oligonucleotide contains one or more template-deficient nucleotides, the template-deficient nucleotides can be at or near the 5' end of the template-deficient oligonucleotide. When the template-deficient oligonucleotide contains two or more template-deficient nucleotides, at least two of the template-deficient nucleotides can be adjacent. In one embodiment, the adjacent template-deficient nucleotides can be within three nucleotides of the 5' end of the template-deficient oligonucleotide. In general, no particular configuration of template-deficient nucleotides is required.

Template-deficient nucleotides are selected from the group consisting of modified nucleotides, derivatized nucleotides, ribonucleotides, and nucleotide analogs. Preferred template-deficient nucleotides are modified nucleotides. Preferred modified nucleotides are abasic nucleotides. Template-deficient nucleotides include abasic nucleotides, nucleotides with an inverted base, fluoro substituted nucleotides, alkyl substituted nucleotides, nucleotides with phenyl substituted ethers, nucleotides with substituted thioethers, nucleotides with phosphate esters, α-nucleotides, 2',3'-dideoxy nucleotides, ribonucleotides, nucleotides derivatized with biotin, nucleotides derivatized with amine,

nucleotides derivatized with Hex, nucleotides derivatized with Tet, nucleotides derivatized with Fam, nucleotides derivatized with fluorescein, nucleotides derivatized with rhodamine, nucleotides derivatized with alkaline phosphatase, nucleotides derivatized with horseradish peroxidase, nucleotides derivatized with spacers, nucleotides derivatized with cholesteryl, nucleotides derivatized with DNP-TEG, nucleotides derivatized with psoralen cross-linkers, nucleotides derivatized with intercalating agents, and nucleotides derivatized with PNA conjugates.

Examples of such nucleotides include abasic nucleosides (Biegelman *et al.*, *Bioorganic & Medicinal Chemistry Letters* 4(14):1715-1720 (1994); Moran *et al.*, *Nucleic Acids Res.* 24(11):2044-2052 (1996); Matray and Kool, *Nature* 399:704-708 (1999)), 5'-fluoro substituted nucleosides (Robins and Wnuk, *Tetrahedron Lett.* 29:5729 (1988)), 5'-alkyl substituted nucleosides (Ray and Jaxa-Chamiec, *Heterocycles* 31(10):1777-1780 (1990); Jun-Dong and Li-He, *Synthesis* 909-911 (1990); Tanaka *et al.*, *Tetrahedron Lett.* 30:2567-2570 (1989)), nucleosides with 5'-alkyl or phenyl substituted ethers (Jones *et al.*, *Carbohydrates, Nucleosides, Nucleotides* 4:301 (1977)), 5'-substituted thioethers (Connolly and Rider, *Nucleic Acids Res.* 13:4485 (1985); Connolly, *Nucleic Acids Res.* 15:3131-3139 (1987); Sinha and Cook, *Nucleic Acids Res.* 16:2659 (1988); Kumar *et al.*, *Nucleic Acids Res.* 19:4561 (1991); Zuckermann *et al.*, *Nucleic Acids Res.* 15:5305 (1987); Gupta *et al.*, Tetrahedron Lett. 31:2471-2474 (1990); Asslie *et al.*, *Tetrahedron* 48:1233-1254 (1992)), 5'-amines and substituted amines (Connolly and Rider, *Nucleic Acids Res.* 13:4485 (1985); Sproat *et al.*, *Nucleic Acids Res.* 15:4857 (1987); Zuckerman *et al.*, *Nucleic Acids Res.* 15:5305 (1987), Li *et al.*, *Nucleic Acids Res.* 15:5275 (1987); Dreyer and Dervan, *Proc. Natl. Acad. Sci. USA* 82:968 (1985)), phosphate esters as 5'-terminators (Tanaka *et al.*, *Tetrahedron Lett.* 30:2567-2570 (1989)), inverted bases or α-nucleosides as 5'-terminators (Bloch *et al.*, *Gene* 72:349 (1988); Sequin, *Helv. Chim. Acta.* 57:68 (1974)), 2',3'-dideoxy nucleosides as 5'-terminators (Huryn and Okabe, *Chem. Rev.* 92:1745-1768 (1992)).

The nucleotides or oligonucleotides can also be derivatized with, for example, biotin, dyes such as fluorescein or rhodamine, or proteins such as alkaline phosphatase or horseradish peroxidase. 5'-modifications useful in the disclosed oligonucleotides include 5'-spacers (Durard *et al.*, *Nucleic Acids Res.* 18:6353 (1990); Salunkhe *et al.*, *J. Amer. Chem. Soc.* 114:8768-8772 (1992); Dolinnaya *et al.*, *Nucleic Acids. Res.* 21:5403-5407 (1993); Takeshita *et al.*, *J. Biol. Chem.* 262:10171-10179 (1987); Kalin *et al.*, *Biochemistry* 27:924-931 (1998)), 5'-biotinylated primers (Cocuzza, *Tetrahedron Lett.* 30:6287-6290 (1989); Nelson *et al.*, *Nucleic Acids Res.* 20:6253-6259 (1992)), 5'-cholesteryl (Mackellar *et al.*, *Nucleic Acids. Res.* 20:3411-3417 (1992); Stein *et al.*, Biochemistry 30:2439-2444 (1991)), 5'-DNP-TEG (Will *et al.*, *Carbohydrate Research* 216:315-322 (1991); Grzybowski *et al.*, *Nucleic Acids Res.* 21:1705-1712 (1993)), 5'-psoralen cross-linkers (Pieles and Englisch, *Nucleic Acids Res.* 17:285 (1989); Taksugi *et al.*, *Proc. Natl. Acad. Sci. USA* 88:5602-5606 (1991)), 5'-intercalating agents (Thoung and Chassignol, *Tetrahedron Lett.* 29:5905 (1988)), 5'-PNA conjugates (Nielsen *et al.*, *Science* 254:1497-1500 (1991); Egholm *et al.*, *J. Am. Chem. Soc.* 114:1895-1897 (1992)), 5'-enzyme conjugates (Jablonski *et al.*, *Nucleic Acids. Res.* 14:6115-6128 (1986)), 5'-dye-label (Molecular Probes, Eugene, Oreg.; Research Organics, Cleveland, Ohio).

It is not required that all of the template-deficient nucleotides in a template-deficient oligonucleotide be the same type of nucleotide. For example, a template-deficient oligonucleotide can include both abasic nucleotides and ribonucleotides as template-deficient nucleotides. Such template-deficient nucleotides are template-deficient for different reasons (the abasic nucleotide has no base to serve as a template while the ribonucleotide is not recognized as a template by the polymerase). There are no restrictions on the combinations of different template-deficient nucleotides that can be used in the same template-deficient oligonucleotide. Similarly, it is not required that all template-deficient oligonucleotides used in a nucleic acid amplification reaction have the same types or patterns of template-deficient nucleotides. For example, a primer

containing abasic nucleotides can be used with a primer containing inverted nucleotides in the same amplification reaction.

The disclosed template-deficient oligonucleotides can be used with any method of nucleic acid amplification. Preferred forms of nucleic acid amplification for use of the disclosed oligonucleotides include nucleic acid amplification reactions involving exponential amplification, either isothermal or with thermal cycling, nucleic acid amplification reactions requiring exponential amplification, either isothermal or with thermal cycling, nucleic acid amplification reactions involving isothermal linear amplification, nucleic acid amplification reactions requiring isothermal linear amplification, nucleic acid amplification reactions involving rolling circle amplification, nucleic acid amplification reactions involving the polymerase chain reaction, and nucleic acid amplification reactions not involving thermal cycling. Examples of nucleic acid amplification reactions are exponential rolling circle amplification (ERCA)(referred to as strand displacement cascade amplification in PCT Application No. WO 97/19193 and as hyperbranched rolling circle amplification in Lizardi *et al.*, *Nature Genetics* 19(3):225-232 (1998)) and rolling circle amplification (RCA)(U.S. Patent No. 5,854,033; PCT Application No. WO 97/19193; Lizardi *et al.*, *Nature Genetics* 19(3):225-232 (1998)); multiple displacement amplification (MDA)(PCT Application WO 99/18241); strand displacement amplification (SDA)(Walker *et al.*, Nucleic Acids Research 20:1691-1696 (1992), Walker *et al.*, Proc. Natl. Acad. Sci. USA 89:392-396 (1992)); nucleic acid sequence based amplification (NASBA)(Compton, *Nature* 350:91-92 (1991)); transcription-mediated amplification (TMA)(Nelson, *Crit Rev Clin Lab Sci* 35:369-414 (1998)); polymerase chain reaction (PCR) and other exponential amplification techniques involving thermal cycling, self-sustained sequence replication (3SR), and amplification with Qβ replicase (Birkenmeyer and Mushahwar, *J. Virological Methods* 35:117-126 (1991); Landegren, *Trends Genetics* 9:199-202 (1993)); various linear amplification techniques involving thermal cycling such as cycle sequencing (Craxton *et al.*,

Methods Companion Methods in Enzymology 3:20-26 (1991)). The disclosed oligonucleotides and method would be useful in techniques described in Lizardi *et al.*, *Nature Genetics* 3:225-232 (1998), Thomas *et al.*, *Arch Pathol Lab Med* 123:1170-1176 (1999), Baner *et al.*, *Nucleic Acids Research* 26:5073-5078 (1998), and Zhang *et al.*, *Gene* 211:277-285 (1998).

When using 3' closed oligonucleotides as primers, it is preferred that the nucleic acid amplification reaction not involve cycle sequencing, the nucleic acid amplification reaction not require linear amplification via thermal cycling, the nucleic acid amplification reaction not involve linear amplification via thermal cycling, or the nucleic acid amplification reaction involve exponential amplification via thermal cycling.

Also disclosed are kits useful for reducing artifacts in nucleic acid amplification reactions. The disclosed kits include a template-deficient oligonucleotide as described herein and a polymerase. Any form of template-deficient primer as disclosed herein can be included in the disclosed kits.

While not wishing to be limited to any particular mechanism, it is believed that non-cycle oligonucleotide-based artifacts could be generated in the following manner. First, mispriming occurs such that, for example, one oligonucleotide partially anneals to a second oligonucleotide and is extended to the end of the oligonucleotide (see Figure 1). This blunt-ended product may sit in the polymerase active site for an extended period of time. The enzyme binding site for the single-stranded template DNA strand is vacant, and then a second oligonucleotide transiently slips into the active site. Such binding may be stabilized by interaction with the amino acids in the enzyme single-stranded template binding site or the stacking energy between the 5' template-strand base and the 3' end interloping-strand base. Such binding would ordinarily not be very stable or long-lived but may be long enough for a nucleotide to be added to the 3' end of the nascent strand. This would further stabilize the interloping oligonucleotide which could then be copied quickly to its full extent. As a result, the enzyme sits with a blunt-ended duplex in its active site, and another

oligonucleotide can then fit into the template-strand binding site and the cycle repeats itself. In this way, long DNA products may be generated. This process of artifactual synthesis of long products by the binding of successive oligonucleotides into the template-strand binding site is referred to herein as templating (see Figure 1).

Definitions

A template-deficient oligonucleotide is an oligonucleotide with at least one region that cannot serve as a template for nucleic acid synthesis. The functional effect is that a template-deficient oligonucleotide cannot be fully replicated. A template-deficient oligonucleotide can be made by, for example, including template-deficient nucleotides in the oligonucleotide. Whether an oligonucleotide is a template-deficient oligonucleotide can depend on the nucleic acid polymerase being used since certain types of nucleotides can be used as a template by some polymerases but not others. For example, many DNA polymerases require a DNA template. Accordingly, as used herein, whether an oligonucleotide is template-deficient is determined based on the nucleic acid polymerase being used in the nucleic acid amplification reaction. Primers that are template-deficient oligonucleotides are referred to herein as template-deficient primers.

Template-deficient nucleotides are nucleotides or nucleotide analogs that (when contained in a nucleic acid molecule) cannot serve as a template for nucleic acid synthesis. Examples of template-deficient nucleotides include abasic nucleotides and derivatized nucleotides. The functional effect is that a template-deficient nucleotide prevents synthesis of a nucleic acid strand complementary to a nucleic acid strand containing a template-deficient nucleotide at or beyond the site of the template-deficient nucleotide. Template-capable nucleotides are nucleotides that are not template-deficient. Whether a nucleotide is a template-deficient nucleotide can depend on the nucleic acid polymerase being used since certain types of nucleotides can be used as a template by some polymerases but not others. For example, many DNA

polymerases require a deoxyribonucleotide template. Accordingly, as used herein, whether a nucleotide is template-deficient is determined based on the nucleic acid polymerase being used in the nucleic acid amplification reaction.

Oligonucleotide-based artifacts are nucleic acids that are formed in a nucleic acid amplification reaction in the absence of nucleic acids other than the oligonucleotides. Oligonucleotide-based artifacts are to be distinguished from artifacts that result from unintended or illegitimate priming of a template nucleic acid (most often a template that is not the intended template). It should be noted that oligonucleotide-based artifacts may occur in the presence of nucleic acids other than the oligonucleotides. The formation of oligonucleotide-based artifacts in the absence of other nucleic acids is used primarily to distinguish oligonucleotide-based artifacts from other types of nucleic acid amplification artifacts. Non-cycle oligonucleotide-based artifacts are oligonucleotide-based artifacts that are formed in nucleic acid amplification reactions not involving thermal cycling. Cycle oligonucleotide-based artifacts are oligonucleotide-based artifacts that are formed in nucleic acid amplification reactions that involve thermal cycling where formation of the artifacts depends on thermal cycling. Primer-based artifacts are oligonucleotide-based artifacts that are formed from or by primers.

Primer-deficient nucleotides are nucleotides or nucleotide analogs that (when contained in a nucleic acid molecule) cannot hybridize (i.e. cannot hydrogen bond) to a complementary nucleotide. Examples of primer-deficient nucleotides include abasic nucleotides and derivatized nucleotides. The functional effect is that a primer-deficient nucleotide prevents formation of a hybrid to a nucleic acid strand containing a primer-deficient nucleotide at the site of the primer-deficient nucleotide. Primer-capable nucleotides are nucleotides that are not primer-deficient. Examples of primer-capable nucleotides are common ribonucleotides and deoxyribonucleotides.

A 3' open oligonucleotide is a template-deficient oligonucleotide where the template-deficient oligonucleotide includes one or more template-deficient

nucleotides, and where the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end of the template-deficient oligonucleotide is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction. A 3' closed oligonucleotide is a template-deficient oligonucleotide where the template-deficient oligonucleotide includes one or more template-deficient nucleotides, where the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end of the template-deficient oligonucleotide is insufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction. Put another way, placing a template-deficient nucleotide at or near the 3' end of an oligonucleotide results in a 3' closed oligonucleotide, while keeping the region of the oligonucleotide at or near the 3' end free of template-deficient nucleotides results in a 3' open oligonucleotide.

As used herein, a nucleic acid amplification reaction is said to "require" a specified component or condition when the amplification reaction would not function or would not result in the desired amplification. For example, PCR requires thermal cycling since PCR amplification depends on separation of synthesized strands and annealing of primers to both template and synthesized strands for another round of replication. As used herein, a nucleic acid amplification reaction is said to "involve" a specified component or condition when the amplification reaction uses the component or condition. Whether a given amplification reaction involves a particular component depends on the specific reaction involved. For example, a PCR reaction may be performed using labeled primers. Such a reaction can thus be said to "involve" labeled primers. However, since PCR can function without such primers, they would not be considered "required" for PCR. All amplification reactions that "require" a given component or condition also "involve" that component or condition, but

not all amplification reactions that "involve" a given component or condition also "require" that component or condition.

Linear amplification refers to nucleic acid amplification that produces, or is designed to produce, an increase in the target nucleic acid directly proportional to the amount of target nucleic acid in the reaction. For example, cycle sequencing produces one strand (or, more precisely, one chain-terminated partial strand) for every target strand present. The amplification is linear since the synthesized strands are not used as templates in subsequent rounds. Exponential amplification refers to nucleic acid amplification that produces, or is designed to produce, an increase in the target nucleic acid geometrically proportional to the amount of target nucleic acid in the reaction. For example, PCR produces one strand for every original target strand and for every synthesized strand present. The amplification is exponential since the synthesized strands are used as templates in subsequent rounds. An amplification reaction need not actually produce exponentially increasing amounts of nucleic acid to be considered exponential amplification, so long as the amplification reaction is designed to produce such increases.

Cycle sequencing refers to nucleic acid sequencing reactions that require thermal cycling. Cycling, in a nucleic acid amplification reaction, refers to a periodic, repeating change in some condition of the reaction. Minor changes in the condition, such as a slight variation in temperature due to temperature-holding limits of a water bath, is not considered to be cycling, especially where such variations do not have a functional effect on the reaction. Thermal cycling, in a nucleic acid amplification reaction, refers to a periodic, repeating change in the temperature of the reaction of the reaction. PCR requires thermal cycling.

A nucleic acid amplification reaction is a reaction that results in synthesis of more than one copy of all or part of one or more nucleic acid molecules. As used herein, a nucleic acid amplification reaction includes assays, reactions, techniques, and procedures that involve nucleic acid amplification. The fact that such assays, reactions, techniques, and procedures

have a goal other than or in addition to nucleic acid amplification--such as nucleic acid sequencing or nucleic acid detection--does not prevent it from being a nucleic acid amplification reaction.

As used herein, nucleoside refers to adenosine, guanosine, cytidine, uridine, 2'-deoxyadenosine, 2'-deoxyguanosine, 2'-deoxycytidine, or thymidine. A nucleoside analog is a chemically modified form of nucleoside containing a chemical modification at any position on the base or sugar portion of the nucleoside. As used herein, the term nucleoside analog encompasses, for example, both nucleoside analogs based on naturally occurring modified nucleosides, such as inosine and pseudouridine, and nucleoside analogs having other modifications, such as modifications to the 2' position of the sugar. As used herein, nucleotide refers to a phosphate derivative of nucleosides as described above, and a nucleotide analog is a phosphate derivative of nucleoside analogs as described above. The subunits of oligonucleotide analogs, such as peptide nucleic acids, are also considered to be nucleotide analogs.

As used herein, a ribonucleotide is a nucleotide having a 2' hydroxyl function. Analogously, a 2'-deoxyribonucleotide is a nucleotide having only 2' hydrogens. Thus, ribonucleotides and deoxyribonucleotides as used herein refer to naturally occurring nucleotides having nucleoside components adenosine, guanosine, cytidine, and uridine, or 2'-deoxyadenosine, 2'-deoxyguanosine, 2'-deoxycytidine, and thymidine, respectively, without any chemical modification. Ribonucleosides, deoxyribonucleosides, ribonucleoside analogs and deoxyribonucleoside analogs are similarly defined except that they lack the phosphate group, or an analog of the phosphate group, found in nucleotides and nucleotide analogs.

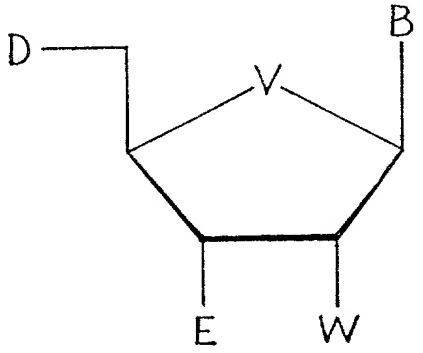
As used herein, oligonucleotide analogs are polymers of nucleic acid-like material with nucleic acid-like properties, such as sequence dependent hybridization, that contain at one or more positions, a modification away from a standard RNA or DNA nucleotide. A preferred example of an oligonucleotide analog is peptide nucleic acid.

The meaning of the above terms is further illustrated by usage of the terms elsewhere herein. The meaning of additional terms used herein but not defined above can generally be understood by common usage in the art and by the context of their usage. The above definitions are not intended to be an exclusive list of terms used and defined herein.

Oligonucleotides

A variety of oligonucleotide structures can be used to create template-deficient oligonucleotides. In general, template-deficient nucleotides useful for making template-deficient oligonucleotides include modified nucleotides, derivatized nucleotides, ribonucleotides, and nucleotide analogs. For example, the oligonucleotide can have one or more abasic nucleotides, one or more nucleotides with an inverted base, fluoro substituted nucleosides, one or more alkyl substituted nucleosides, one or more nucleosides with alkyl groups, one or more nucleoside with phenyl substituted ethers, one or more nucleotides with substituted thioethers, one or more nucleosides with phosphate esters, one or more α-nucleosides, one or more 2',3'-dideoxy nucleosides, or one or more nucleotides derivatized with compounds such as biotin, amine, Hex, Tet, Fam, fluorescein, rhodamine, alkaline phosphatase, horseradish peroxidase, spacers, cholesteryl, DNP-TEG, psoralen cross-linkers, intercalating agents, PNA conjugates, other enzyme conjugates, and other dye-labels.

One class of modified or derivatized nucleotides have the structure:



Each B can be -H, -OH, -COOH, $-CONH_2$, $-CONHR^1$, $-CONR^1R^2$, $-NH_2$, $-NHR^1$, $-NR^1R^2$, $-NHCOR^1$, -SH, SR^1, -F, $-ONH_2$, $-ONHR^1$, $-ONR^1R^2$, -

NHOH, -NHOR[1], -NR[2]OH, -NR[2]OR[1], substituted or unsubstituted C_1-C_{10} straight chain or branched alkyl, substituted or unsubstituted C_2-C_{10} straight chain or branched alkenyl, substituted or unsubstituted C_2-C_{10} straight chain or branched alkynyl, substituted or unsubstituted C_1-C_{10} straight chain or branched alkoxy, substituted or unsubstituted C_2-C_{10} straight chain or branched alkenyloxy, and substituted or unsubstituted C_2-C_{10} straight chain or branched alkynyloxy. The substituents for W groups are independently halogen, cyano, amino, carboxy, ester, ether, carboxamide, hydroxy, or mercapto. R^1 and R^2 can be substituted or unsubstituted alkyl, alkenyl, or alkynyl groups, where the substituents are independently halogen, cyano, amino, carboxy, ester, ether, carboxamide, hydroxy, or mercapto.

Each V can be an O, S, NH, or CH_2 group.

Each W can be -H, -OH, -COOH, -$CONH_2$, -$CONHR^1$, -$CONR^1R^2$, -NH_2, -NHR^1, -NR^1R^2, -NHCOR[1], -SH, SR^1, -F, -ONH_2, -$ONHR^1$, -ONR^1R^2, -NHOH, -$NHOR^1$, -NR^2OH, -NR^2OR^1, substituted or unsubstituted C_1-C_{10} straight chain or branched alkyl, substituted or unsubstituted C_2-C_{10} straight chain or branched alkenyl, substituted or unsubstituted C_2-C_{10} straight chain or branched alkynyl, substituted or unsubstituted C_1-C_{10} straight chain or branched alkoxy, substituted or unsubstituted C_2-C_{10} straight chain or branched alkenyloxy, and substituted or unsubstituted C_2-C_{10} straight chain or branched alkynyloxy. The substituents for W groups are independently halogen, cyano, amino, carboxy, ester, ether, carboxamide, hydroxy, or mercapto. R^1 and R^2 can be substituted or unsubstituted alkyl, alkenyl, or alkynyl groups, where the substituents are independently halogen, cyano, amino, carboxy, ester, ether, carboxamide, hydroxy, or mercapto.

D and E are residues which together form a phosphodiester or phosphorothioate diester bond between adjacent nucleosides or nucleoside analogues or together form an analogue of an internucleosidic bond.

All of the above modifications may make it difficult for a polymerase to add nucleotides opposite such modified nucleotides or extend synthesis beyond

such modified nucleotides. A combination of nucleotides having different modifications, derivatizations, or substitutions can be used in a single template-deficient oligonucleotide. Similarly, primers having different types of modifications, derivatizations, or substitutions can be used in the same amplification reaction (see Example 2 and Figure 7).

Template-deficient oligonucleotides can also be made without using modified nucleotides, derivatized nucleotides, or nucleotide analogs. For example, ordinary ribonucleotides can be used as template-deficient nucleotides, depending on the polymerase used. Polymerases that require a DNA template will not be able to use ribonucleotides in the primer.

It is preferred that template-deficient oligonucleotides have at least two consecutive or adjacent template-deficient nucleotides. Such an arrangement reduces the chance that synthesis could pass over a template-deficient region in the oligonucleotide. For example, some polymerases, such as Taq DNA polymerase, add an overhanging 3' nucleotide to a blunt-ended double-stranded DNA structure. This might overhang the 5' abasic base in the templating scenario and allow templating to occur anyway. Use of two consecutive abasic nucleotides should inhibit synthesis across the nick (see Figure 3). The examples shows the reduction in artifacts in an ERCA reaction when two primer oligonucleotides, P1 and P2, each with two 5' abasic nucleotides are used (see Figure 6).

A subset of the disclosed oligonucleotides are preferred for use in amplification reactions involving thermal cycling, such as PCR. Unlike the primers of Stump *et al.*--which have few enough template-capable nucleotides 3' of the most 3' template-deficient nucleotide to prevent synthesis of a strand to which the primer can effectively prime--the subset of the disclosed primers preferred for thermal cycling reactions will have enough template-capable nucleotides 3' of the most 3' template-deficient nucleotide to allow effective priming of synthesized strands.

Template-deficient nucleotides can be placed anywhere in an oligonucleotide. It may be desirable to keep the 5' end of an oligonucleotide free of template-deficient nucleotides. For example, oligonucleotides with 5' abasic residues or other modifications may not be effectively labeled by phosphorylation using T4 polynucleotide kinase and γ^{32}P-ATP.

Method

The disclosed method involves using one or more of the disclosed template-deficient oligonucleotides in a nucleic acid amplification reaction of interest. Most commonly, the disclosed oligonucleotides will be used as primers in the amplification reaction of interest. Where more than one oligonucleotide is needed or used in the reaction, it is preferred that all of the oligonucleotides in the reaction be template-deficient primers. As used herein, a nucleic acid amplification reaction includes assays, reactions, techniques, and procedures that involve nucleic acid amplification.

The advantage of a nucleic acid amplification reaction that is not susceptible artifacts is that the nucleic acid synthesis products will be specific for the input or target template. In particular, false positives in detection assays involving nucleic acid amplification--caused by artifactual nucleic acid synthesis not dependent on the presence of the target sequence--will be reduced. A preferred nucleic acid amplification reaction for use with the disclosed method if exponential rolling circle amplification (ERCA). The advantage of an ERCA reaction that is not susceptible to primer artifacts is that the DNA synthesis products will be specific for the input template and the yield of DNA synthesis products will be in direct proportion to the quantity of input template.

The disclosed method differs from the method described in Stump *et al.*, *Nucleic Acids Research* 27:4642-4648 (1999). In Stump *et al.*, the artifact resulted from the thermal cycling in a PCR reaction. This PCR artifact resulted because the product of one round of replication was a template for subsequent rounds. Such artifacts are referred to herein as cycle oligonucleotide artifacts. Stump *et al.* eliminated the cycle oligonucleotide artifact by using primers that

could not be fully replicated such that the synthesized strand did not contain enough sequence complementary to the primer to allow the primer to prime synthesis on the synthesized strand (these primers are 3' closed oligonucleotides as defined herein). In contrast, 3' open oligonucleotides as defined herein do allow synthesis of a strand that contains enough sequence complementary to the primer to allow the primer to prime synthesis on the synthesized strand. Further, such primers do not yield a blunt, duplex DNA end such as is required for the templating artifact (that is, a non-cycle oligonucleotide artifact) to occur during the isothermal amplification reactions.

The disclosed method is an improvement of nucleic acid amplification reactions making them more specific. In particular, the disclosed method improves the results when ERCA is used for all applications that ERCA may be used for, such as *in vitro* diagnostics and SNP analysis in a multi-well format. The disclosed method may also yield an improvement of PCR and other amplification methods involving thermal cycling, and can be used with any PCR or other thermal cycling technique. For use in PCR and other amplification reactions involving thermal cycling, it is preferred that the primer be a 3' open oligonucleotide (that is, an oligonucleotide that includes a sufficient number of template-capable nucleotides at the 3' end of the primer to allow synthesis of a complement to a sufficient portion of the primer to allow efficient priming by the primer under the amplification conditions used).

Examples

Example 1: Elimination of Primer Artifacts in Exponential Rolling Circle Amplification Using Primers With Abasic Nucleotides

This example demonstrates that significant primer artifacts are generated in ERCA when no template is present, and that template-deficient primers having two abasic nucleotides at the 5' end eliminate these primer artifacts. As with most nucleic acid amplification techniques used to detect specific nucleic acids, artifactual production of spurious nucleic acids in the absence of the

nucleic acid to be detected can result in false positive assays. In assays where the nucleic acid to be detected is present, artifactual production of spurious nucleic acids can affect the accuracy of the assay both qualitatively and, most significantly, quantitatively. This example demonstrates the elimination of such artifacts.

Ten reactions were carried out under the conditions used for ERCA in order to illustrate the reduction of primer-based artifacts by using primers containing two template-deficient nucleotides at the 5' ends. Reactions (30 μl) contained 20 mM Tris-HCl, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM MgSO4, 0.1% Triton X-100 (pH 8.8 at 25°C). In addition, reactions contained 400 μM deoxyribonucleoside triphosphates, α-[^{32}P] dCTP, specific activity 169 cpm/pmol total dNTP, and 8 units Bst DNA polymerase. ERCA primers were added as indicated, where 'aba' indicates the presence of an abasic nucleotide residue.

P1 (5' end template-capable)

5' CTA AAG CTG AGA CAT GAC GAG TC 3' (SEQ ID NO:1)

P2 (5' end template-capable)

5' GTG ATT CCA CCT TCT CC 3' (SEQ ID NO:2)

P1aba (5' end template-deficient)

5' (aba)(aba) CTA AAG CTG AGA CAT GAC GAG TC 3' (SEQ ID NO:3)

P2aba (5' end template-deficient)

5' (aba)(aba) GTG ATT CCA CCT TCT CC 3' (SEQ ID NO:4)

Reactions contained single-stranded circular ERCA template DNA designated 'G542X gap padlock circle' as indicated, prepared as described by Lizardi *et al.*, *Nature Genetics* 3:225-232 (1998).

Reaction	Additions
1	P1 + P2 + G542X gap padlock circle
2	P1 + P2
3	P1

	4	P2
	5	P1aba + P2aba + G542X gap padlock circle
	6	P1aba + P2aba
	7	P1aba
5	8	P2aba
	9	P1 + P2aba
	10	P1aba + P2

Reactions were assembled on ice and incubated for 30 minutes on ice. Reactions were initiated by placing them at 65°C and incubated further for 3 hours at 65°C. Aliquots (4 µl) were taken at 0.5, 1, 1.5, 2, and 3 hours and spotted onto DE81 filter paper in order to quantitate DNA synthesis by the incorporation of radioactive deoxyribonucleotide. The results are shown in Figure 6.

As can be seen, significant artifactual DNA production occurs in the absence of template when using template-capable primers (see P1 + P2 - circle curve). Such artifactual DNA is eliminated when template-deficient primers are used (see P1(aba) + P2(aba) - circle curve). The use of template-deficient primers does not prevent legitimate amplification (see P1(aba) + P2(aba) + circle curve). Use of one template-deficient primer and one template-capable primer still results in artifacts, but at a reduced level (see P1 + P2(aba) - circle and P1(aba) + P2 - circle curves).

Example 2: Elimination of Primer Artifacts in Exponential Rolling Circle Amplification Using Primers With Abasic Nucleotides, Ribonucleotides

This example demonstrates that that template-deficient primers having different types of template-deficient nucleotides, used either alone or in combination, can eliminate primer artifacts of ERCA.

Nine reactions were carried out under the conditions described in Example 1. ERCA primers were added as indicated. P1C and P4C are chimeric RNA/DNA oligonucleotides, having 2'-O-methyl ribonucleotides at the 5' end,

as indicated by the use of lower case letters in the sequence. There are 20 2'-O-methyl ribonucleotides at the 5' end of P1C and 18 2'-O-methyl ribonucleotides at the 5' end of P4C. The six nucleotides at the 3' end of P1C and P4C are deoxyribonucleotides, as indicated by the use of capital letters in the sequence.

P1C (5' end template-deficient)

5' aaactaaagctgagacatga CGAGTC 3' (SEQ ID NO:5)

P4C (5' end template-deficient)

5' agtttaatacgactcact ATAGGG 3' (SEQ ID NO:6)

Reaction	Additions
1	P1 + P2 + G542X gap padlock circle
2	P1 + P2
3	P1aba + P2aba + G542X gap padlock circle
4	P1aba + P2aba
5	P1C + P4C
6	P1 + P4C
7	P1C + P2
8	P1aba + P4C
9	P1C + P2aba

Reactions were assembled on ice and incubated for 30 minutes on ice. Reactions were initiated by placing them at 65°C and incubated further for 3 hours at 65°C. Aliquots (4 1) were taken at 0.5, 1, 2, and 3 hours and spotted onto DE81 filter paper in order to quantitate DNA synthesis by the incorporation of radioactive deoxyribonucleotide. These primers were used both in matched pairs (normal with normal, abasic with abasic, chimeric with chimeric) and in mismatched pairs (abasic with chimeric, abasic with normal, chimeric with normal). The results are shown in Figure 7.

As can be seen, all combinations of template-deficient primers prevent artifact formation (see P1aba + P2aba, P1C + P4C, P1aba + P4C and P1C +

P2aba curves). This includes combinations of template-deficient primers having different types of template-deficient nucleotides (see P1aba + P4C and P1C + P2aba curves). Use of one template-deficient primer and one template-capable primer can still results in artifacts (see P1C + P2 curve), but not always (see P1 + P4C curve).

The results are summarized in the following table.

Primer Pair	Non-specific DNA Synthesis
P1 + P2	yes
P1aba + P2aba	no
P1C + P4C	no
P1 + P4C	no
P1C + P2	yes (reduced)
P1aba + P4C	no
P1C + P2aba	no

It is understood that the disclosed invention is not limited to the particular methodology, protocols, and reagents described as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a ", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a host cell" includes a plurality of such host cells, reference to "the antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or

testing of the present invention, the preferred methods, devices, and materials are as described. Publications cited herein and the material for which they are cited are specifically incorporated by reference. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

I claim:

1. A method of reducing formation of artifacts in a nucleic acid amplification reaction, the method comprising

using a template-deficient oligonucleotide as at least one of the oligonucleotides in the nucleic acid amplification reaction,

wherein the template-deficient oligonucleotide comprises one or more template-deficient nucleotides,

wherein the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end of the template-deficient oligonucleotide is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction.

2. The method of claim 1 wherein the one or more template-deficient nucleotides are at or near the 5' end of the template-deficient oligonucleotide.

3. The method of claim 1 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are adjacent.

4. The method of claim 3 wherein the two or more adjacent template-deficient nucleotides are within three nucleotides of the 5' end of the template-deficient oligonucleotide.

5. The method oligonucleotide of claim 1 wherein the template-deficient nucleotides are selected from the group consisting of modified nucleotides, derivatized nucleotides, ribonucleotides, and nucleotide analogs.

6. The method oligonucleotide of claim 1 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are different.

7. The method of claim 1 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the

two or more template-deficient nucleotides are template-deficient for different reasons.

8. The method of claim 5 wherein the template-deficient nucleotides are modified nucleotides.

9. The method of claim 5 wherein the modified nucleotides are abasic nucleotides.

10. The method of claim 5 wherein the template-deficient nucleotides are selected from the group consisting of abasic nucleotides, nucleotides with an inverted base, fluoro substituted nucleotides, alkyl substituted nucleotides, nucleotides with phenyl substituted ethers, nucleotides with substituted thioethers, nucleotides with phosphate esters, α-nucleotides, 2',3'-dideoxy nucleotides, ribonucleotides, nucleotides derivatized with biotin, nucleotides derivatized with amine, nucleotides derivatized with Hex, nucleotides derivatized with Tet, nucleotides derivatized with Fam, nucleotides derivatized with fluorescein, nucleotides derivatized with rhodamine, nucleotides derivatized with alkaline phosphatase, nucleotides derivatized with horseradish peroxidase, nucleotides derivatized with spacers, nucleotides derivatized with cholesteryl, nucleotides derivatized with DNP-TEG, nucleotides derivatized with psoralen cross-linkers, nucleotides derivatized with intercalating agents, and nucleotides derivatized with PNA conjugates.

11. The method of claim 1 wherein the nucleic acid amplification reaction does not involve cycle sequencing.

12. The method of claim 11 wherein the nucleic acid amplification reaction does not require linear amplification via thermal cycling.

13. The method of claim 12 wherein the nucleic acid amplification reaction does not involve linear amplification via thermal cycling.

14. The method of claim 1 wherein the nucleic acid amplification reaction involves exponential amplification via thermal cycling.

15. The method of claim 14 wherein the nucleic acid amplification reaction requires exponential amplification via thermal cycling.

16. The method of 14 wherein the nucleic acid amplification reaction involves the polymerase chain reaction.

17. The method of claim 1 wherein the nucleic acid amplification does not involve thermal cycling.

18. The method of 17 wherein the nucleic acid amplification is rolling circle amplification.

19. The method of claim 1 wherein the nucleic acid amplification reaction is selected from the group consisting of exponential rolling circle amplification (ERCA), and rolling circle amplification (RCA), multiple displacement amplification (MDA), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), transcription-mediated amplification (TMA), polymerase chain reaction (PCR), self-sustained sequence replication (3SR), amplification with Qβ replicase, and cycle sequencing.

20. The method of claim 1 wherein the template-deficient oligonucleotide is a primer.

21. The method of claim 20 wherein all of the primers used in the nucleic acid amplification reaction are template-deficient.

22. The method of claim 1 wherein all of the oligonucleotides used in the nucleic acid amplification reaction are template-deficient.

23. A method of reducing formation of artifacts in a nucleic acid amplification reaction, the method comprising

using a template-deficient oligonucleotide as at least one of the oligonucleotides in the nucleic acid amplification reaction,

wherein the nucleic acid amplification reaction does not involve cycle sequencing.

24. The method of claim 23 wherein the nucleic acid amplification reaction does not require linear amplification via thermal cycling.

25. The method of claim 24 wherein the nucleic acid amplification reaction does not involve linear amplification via thermal cycling.

26. The method of claim 23 wherein the nucleic acid amplification does not involve thermal cycling.

27. The method of 26 wherein the nucleic acid amplification is rolling circle amplification.

28. The method of claim 23 wherein the nucleic acid amplification reaction involves exponential amplification via thermal cycling.

29. The method of claim 28 wherein the nucleic acid amplification reaction requires exponential amplification via thermal cycling.

30. The method of 28 wherein the nucleic acid amplification reaction involves the polymerase chain reaction.

31. The method of claim 23 wherein the nucleic acid amplification reaction is selected from the group consisting of exponential rolling circle amplification (ERCA), and rolling circle amplification (RCA), multiple displacement amplification (MDA), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), transcription-mediated amplification (TMA), polymerase chain reaction (PCR), self-sustained sequence replication (3SR), and amplification with Qβ replicase.

32. The method of claim 23 wherein the template-deficient oligonucleotide comprises one or more template-deficient nucleotides.

33. The method of claim 32 wherein the one or more template-deficient nucleotides are at or near the 5' end of the template-deficient oligonucleotide.

34. The method of claim 32 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are adjacent.

35. The method of claim 34 wherein the two or more adjacent template-deficient nucleotides are within three nucleotides of the 5' end of the template-deficient oligonucleotide.

36. The method oligonucleotide of claim 32 wherein the template-deficient nucleotides are selected from the group consisting of modified nucleotides, derivatized nucleotides, ribonucleotides, and nucleotide analogs.

37. The method oligonucleotide of claim 32 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are different.

38. The method of claim 32 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are template-deficient for different reasons.

39. The method of claim 36 wherein the template-deficient nucleotides are modified nucleotides.

40. The method of claim 36 wherein the modified nucleotides are abasic nucleotides.

41. The method of claim 36 wherein the template-deficient nucleotides are selected from the group consisting of abasic nucleotides, nucleotides with an inverted base, fluoro substituted nucleotides, alkyl substituted nucleotides, nucleotides with phenyl substituted ethers, nucleotides with substituted thioethers, nucleotides with phosphate esters, α-nucleotides, 2',3'-dideoxy nucleotides, ribonucleotides, nucleotides derivatized with biotin, nucleotides derivatized with amine, nucleotides derivatized with Hex, nucleotides derivatized with Tet, nucleotides derivatized with Fam, nucleotides derivatized with fluorescein, nucleotides derivatized with rhodamine, nucleotides derivatized with alkaline phosphatase, nucleotides derivatized with horseradish peroxidase, nucleotides derivatized with spacers, nucleotides derivatized with cholesteryl, nucleotides derivatized with DNP-TEG, nucleotides derivatized with psoralen cross-linkers, nucleotides derivatized with intercalating agents, and nucleotides derivatized with PNA conjugates.

42. The method of claim 32 wherein the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction.

43. The method of claim 23 wherein the template-deficient oligonucleotide is a primer.

44. The method of claim 43 wherein all of the primers used in the nucleic acid amplification reaction are template-deficient.

45. The method of claim 23 wherein all of the oligonucleotides used in the nucleic acid amplification reaction are template-deficient.

46. A method of reducing formation of artifacts in a nucleic acid amplification reaction, the method comprising

using a template-deficient oligonucleotide as at least one of the oligonucleotides in the nucleic acid amplification reaction,

wherein the nucleic acid amplification reaction involves exponential amplification via thermal cycling.

47. The method of claim 46 wherein the nucleic acid amplification reaction requires exponential amplification via thermal cycling.

48. The method of 46 wherein the nucleic acid amplification reaction involves the polymerase chain reaction.

49. The method of claim 46 wherein the template-deficient oligonucleotide comprises one or more template-deficient nucleotides.

50. A template-deficient oligonucleotide comprising one or more template-deficient nucleotides,

wherein the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction.

51. The template-deficient oligonucleotide of claim 50 wherein the one or more template-deficient nucleotides are at or near the 5' end of the template-deficient oligonucleotide.

52. The template-deficient oligonucleotide of claim 50 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are adjacent.

53. The template-deficient oligonucleotide of claim 52 wherein the two or more adjacent template-deficient nucleotides are within three nucleotides of the 5' end of the template-deficient oligonucleotide.

54. The template-deficient oligonucleotide of claim 50 wherein the template-deficient nucleotides are selected from the group consisting of modified nucleotides, derivatized nucleotides, ribonucleotides, and nucleotide analogs.

55. The template-deficient oligonucleotide of claim 50 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are different.

56. The template-deficient oligonucleotide of claim 50 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are template-deficient for different reasons.

57. The template-deficient oligonucleotide of claim 54 wherein the template-deficient nucleotides are modified nucleotides.

58. The template-deficient oligonucleotide of claim 54 wherein the modified nucleotides are abasic nucleotides.

59. The template-deficient oligonucleotide of claim 54 wherein the template-deficient nucleotides are selected from the group consisting of abasic nucleotides, nucleotides with an inverted base, fluoro substituted nucleotides, alkyl substituted nucleotides, nucleotides with phenyl substituted ethers, nucleotides with substituted thioethers, nucleotides with phosphate esters, α-nucleotides, 2',3'-dideoxy nucleotides, ribonucleotides, nucleotides derivatized with biotin, nucleotides derivatized with amine, nucleotides derivatized with Hex, nucleotides derivatized with Tet, nucleotides derivatized with Fam, nucleotides derivatized with fluorescein, nucleotides derivatized with rhodamine, nucleotides derivatized with alkaline phosphatase, nucleotides derivatized with horseradish peroxidase, nucleotides

derivatized with spacers, nucleotides derivatized with cholesteryl, nucleotides derivatized with DNP-TEG, nucleotides derivatized with psoralen cross-linkers, nucleotides derivatized with intercalating agents, and nucleotides derivatized with PNA conjugates.

60. A kit for nucleic acid amplification, the kit comprising

a template-deficient primer, wherein the template-deficient primer comprises one or more template-deficient nucleotides,

wherein the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction.

61. The kit of claim 60 wherein the one or more template-deficient nucleotides are at or near the 5' end of the template-deficient primer.

62. The kit of claim 60 wherein the template-deficient primer comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are adjacent.

63. The kit of claim 62 wherein the two or more adjacent template-deficient nucleotides are within three nucleotides of the 5' end of the template-deficient primer.

64. The kit of claim 60 wherein the template-deficient nucleotides are selected from the group consisting of modified nucleotides, derivatized nucleotides, ribonucleotides, and nucleotide analogs.

65. The kit of claim 60 wherein the template-deficient primer comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are different.

66. The kit of claim 60 wherein the template-deficient primer comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are template-deficient for different reasons.

67. The kit of claim 64 wherein the template-deficient nucleotides are modified nucleotides.

68. The kit of claim 64 wherein the modified nucleotides are abasic nucleotides.

69. The kit of claim 64 wherein the template-deficient nucleotides are selected from the group consisting of abasic nucleotides, nucleotides with an inverted base, fluoro substituted nucleotides, alkyl substituted nucleotides, nucleotides with phenyl substituted ethers, nucleotides with substituted thioethers, nucleotides with phosphate esters, α-nucleotides, 2',3'-dideoxy nucleotides, ribonucleotides, nucleotides derivatized with biotin, nucleotides derivatized with amine, nucleotides derivatized with Hex, nucleotides derivatized with Tet, nucleotides derivatized with Fam, nucleotides derivatized with fluorescein, nucleotides derivatized with rhodamine, nucleotides derivatized with alkaline phosphatase, nucleotides derivatized with horseradish peroxidase, nucleotides derivatized with spacers, nucleotides derivatized with cholesteryl, nucleotides derivatized with DNP-TEG, nucleotides derivatized with psoralen cross-linkers, nucleotides derivatized with intercalating agents, and nucleotides derivatized with PNA conjugates.

70. The kit of claim 60 wherein the nucleic acid amplification reaction does not involve cycle sequencing.

71. The kit of claim 70 wherein the nucleic acid amplification reaction does not require linear amplification via thermal cycling.

72. The kit of claim 74 wherein the nucleic acid amplification reaction does not involve linear amplification via thermal cycling.

73. The kit of claim 63 wherein the nucleic acid amplification reaction involves exponential amplification via thermal cycling.

74. The kit of claim 76 wherein the nucleic acid amplification reaction requires exponential amplification via thermal cycling.

75. The kit of claim 76 wherein the nucleic acid amplification reaction involves the polymerase chain reaction.

76. The kit of claim 63 wherein the nucleic acid amplification reaction is selected from the group consisting of exponential rolling circle amplification (ERCA), and rolling circle amplification (RCA), multiple displacement amplification (MDA), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), transcription-mediated amplification (TMA), polymerase chain reaction (PCR), self-sustained sequence replication (3SR), and amplification with Qβ replicase.

METHOD FOR REDUCING ARTIFACTS IN NUCLEIC ACID AMPLIFICATION

ABSTRACT OF THE DISCLOSURE

Disclosed are compositions and methods useful for reducing the formation of artifacts during nucleic acid amplification reactions. The method uses special oligonucleotides, referred to herein as template-deficient oligonucleotides, that cannot serve as a template for nucleic acid synthesis over part of their length. This prevents the oligonucleotides from serving as effective templates in the formation of artifacts. The disclosed method involves using a template-deficient oligonucleotide as at least one of the oligonucleotides (preferably a primer) in a nucleic acid amplification reaction, where the template-deficient oligonucleotide comprises one or more template-deficient nucleotides, preferably at or near the 5' end of the template-deficient oligonucleotide. The disclosed method is useful for reducing artifacts in any nucleic acid amplification reaction involving oligonucleotides. In a preferred form of the method the nucleic acid amplification reaction does not involve thermal cycling. The disclosed method is effective at reducing non-cycle oligonucleotide-based artifacts. Also disclosed are kits useful for reducing artifacts in nucleic acid amplification reactions. The disclosed kits include a template-deficient oligonucleotide, wherein the template-deficient oligonucleotide comprises one or more template-deficient nucleotides, and a nucleic acid polymerase.

Figure 1.

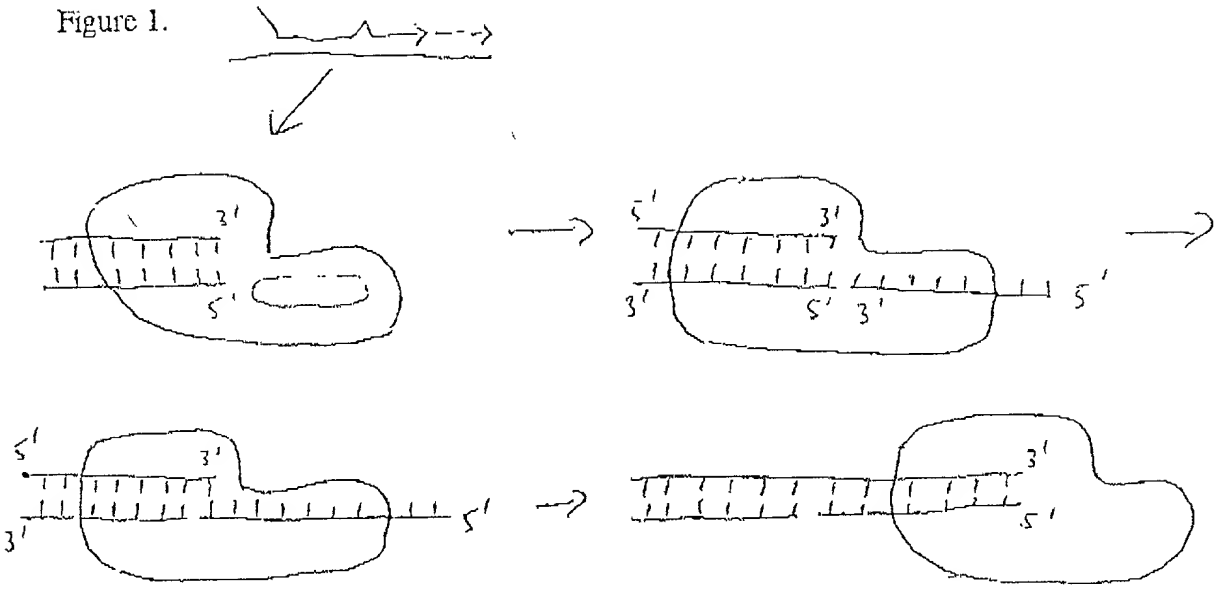


Figure 2.

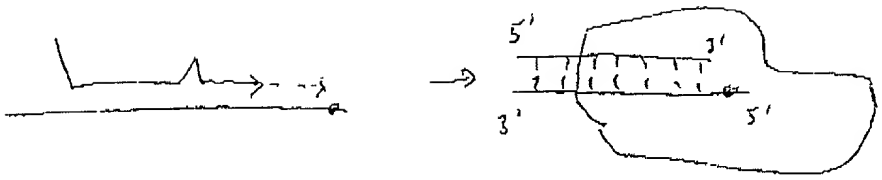


Figure 3.

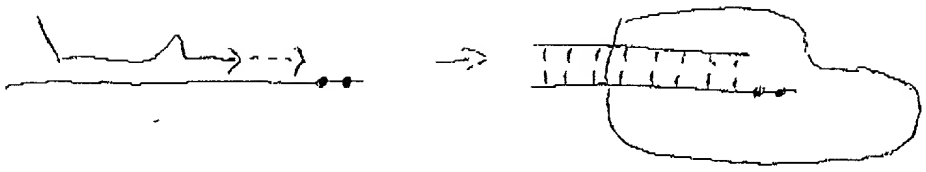


Figure 4.

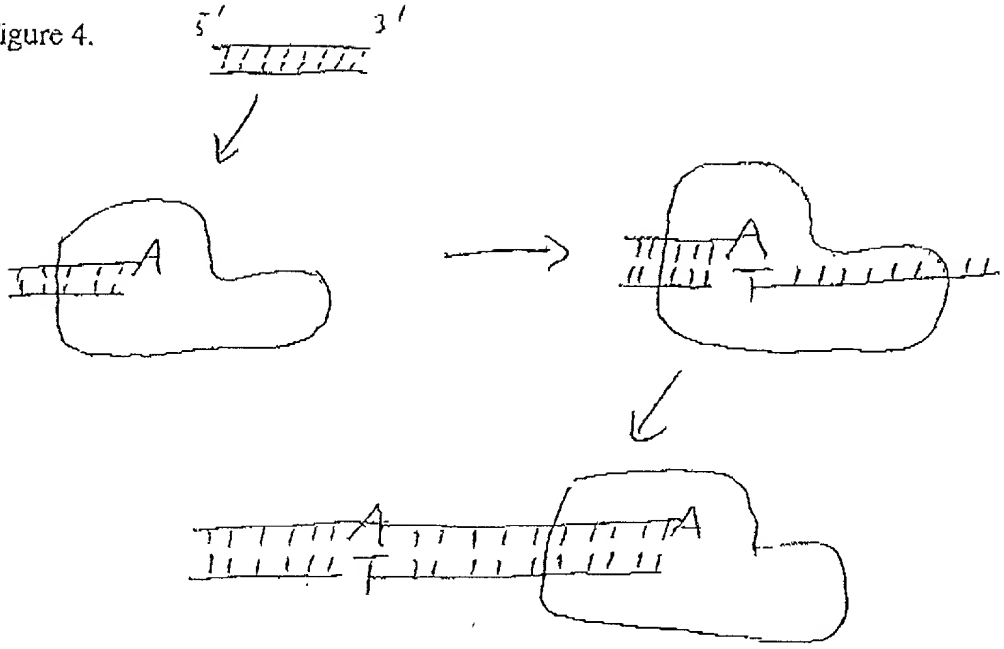


Figure 5

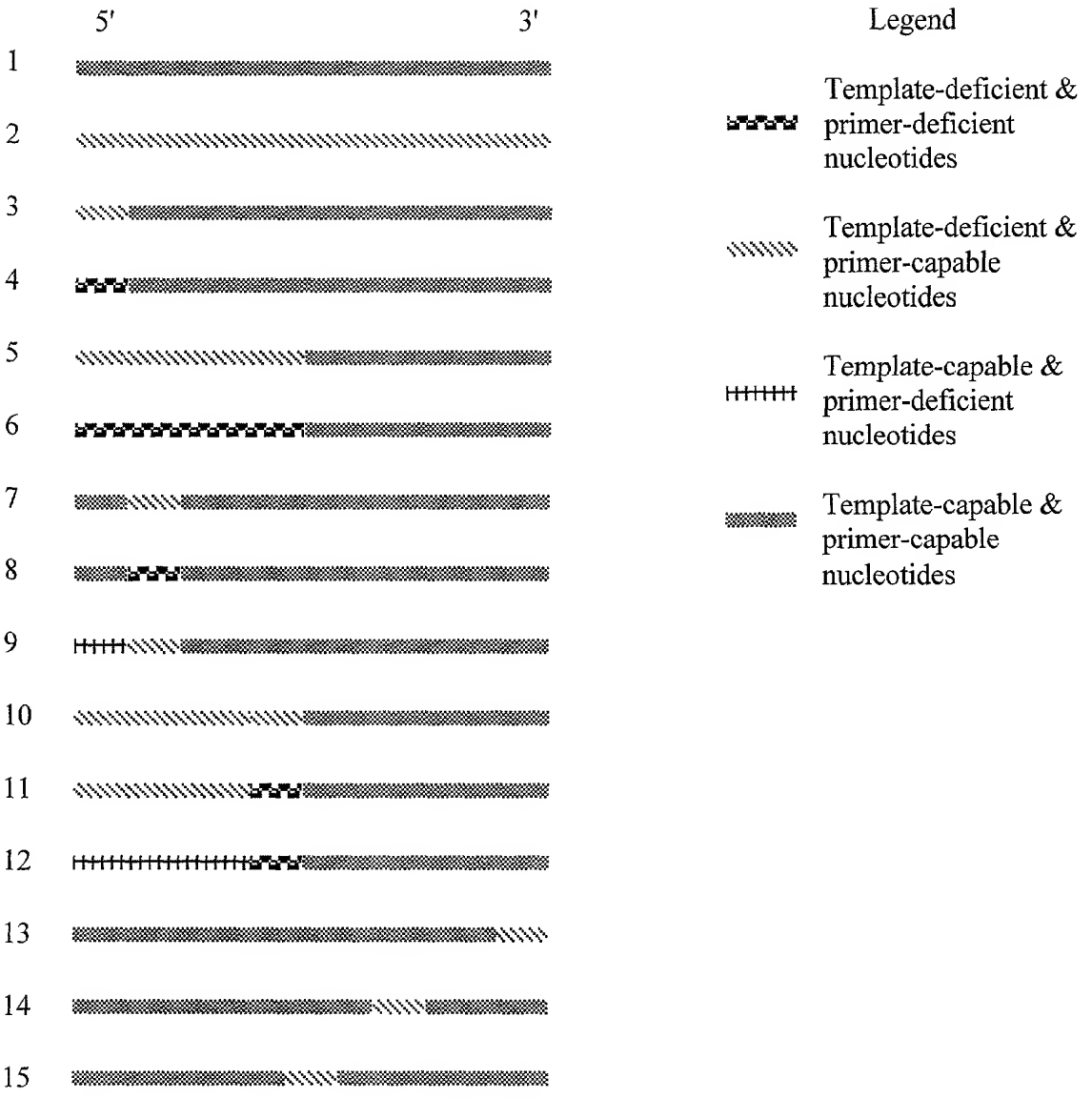

5'
3'
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Legend
Template-deficient & primer-deficient nucleotides
Template-deficient & primer-capable nucleotides
Template-capable & primer-deficient nucleotides
Template-capable & primer-capable nucleotides

Figure 6

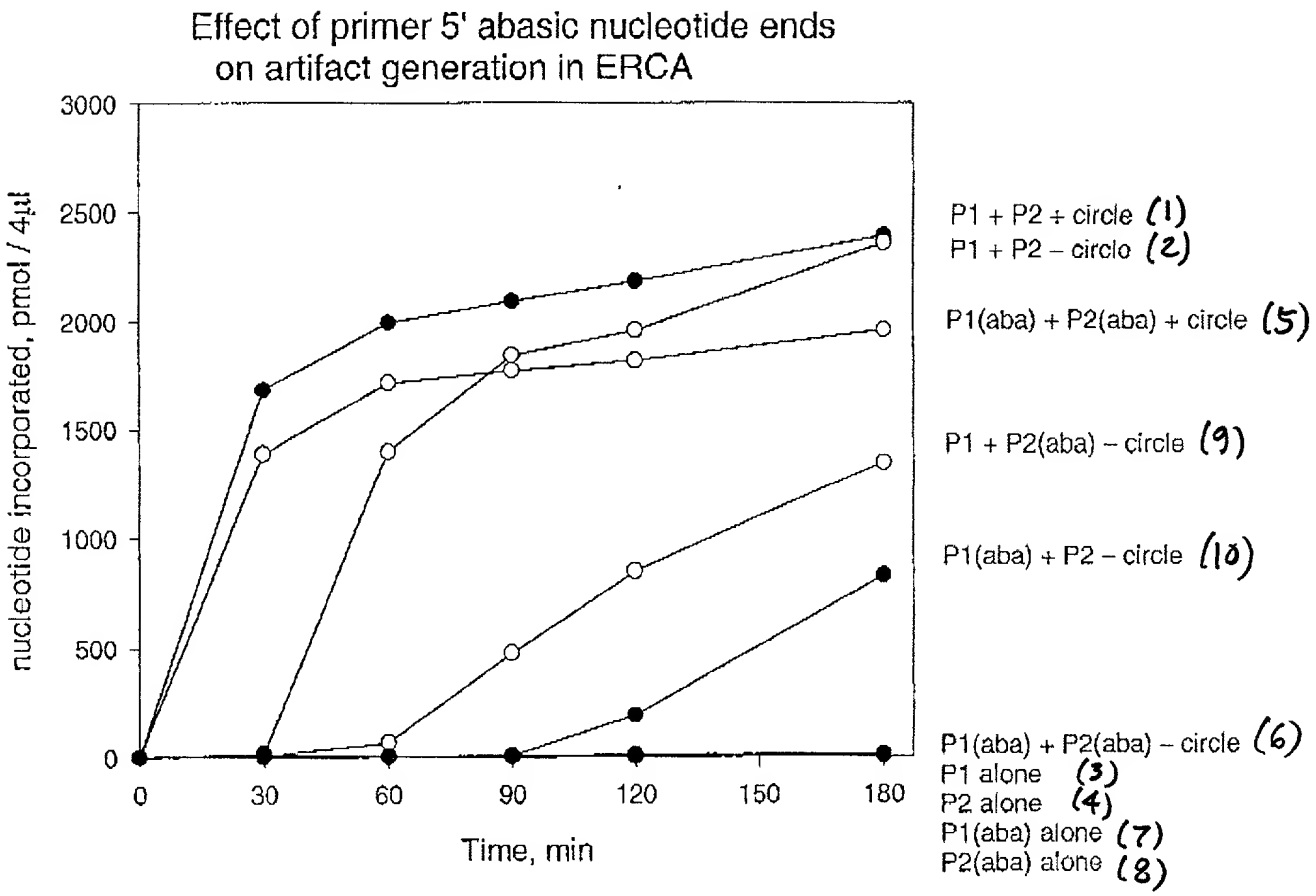
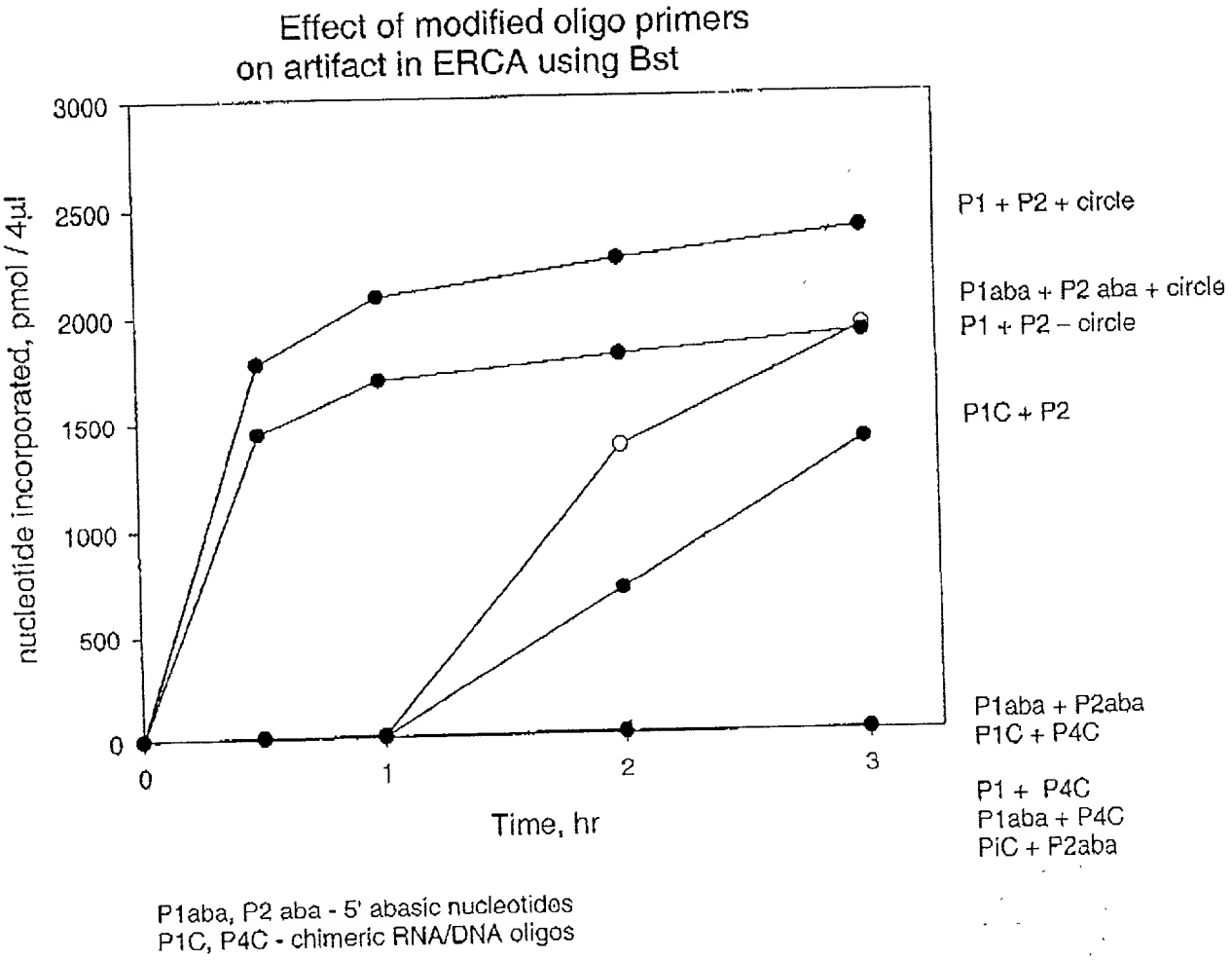

Effect of primer 5' abasic nucleotide ends
on artifact generation in ERCA
nucleotide incorporated, pmol / 4µl
3000
2500
2000
1500
1000
500
0
0
30
60
90
120
150
180
Time, min
P1 + P2 + circle (1)
P1 + P2 – circle (2)
P1(aba) + P2(aba) + circle (5)
P1 + P2(aba) – circle (9)
P1(aba) + P2 – circle (10)
P1(aba) + P2(aba) – circle (6)
P1 alone (3)
P2 alone (4)
P1(aba) alone (7)
P2(aba) alone (8)

Figure 7



P1aba, P2 aba - 5' abasic nucleotides
P1C, P4C - chimeric RNA/DNA oligos

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☒ Declaration Submitted with Initial Filing **OR** ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	MSI 100
First Named Inventor	Frank B. Dean
COMPLETE IF KNOWN	
Application Number	/
Filing Date	February 25, 2000
Group Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR REDUCING ARTIFICATS IN NUCLEIC ACID AMPLIFICATION

(Title of the Invention)

the specification of which

☒ is attached hereto

OR

☐ was filed on (MM/DD/YYYY) _____ as United States Application Number or PCT International Application Number _____ and was amended on (MM/DD/YYYY) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)

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DECLARATION — Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number *(if applicable)*

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As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: ☐ Customer Number [ ] → *Place Customer Number Bar Code Label here*
OR
☒ Registered practitioner(s) name/registration number listed below

Name	Registration Number	Name	Registration Number
Patrea L. Pabst	31,284		
Robert A. Hodges	41,074		
Kevin W. King	42,737		

☐ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

Direct all correspondence to: ☐ Customer Number or Bar Code Label [ ] *OR* ☒ Correspondence address below

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
Frank B.	Dean

Inventor's Signature						Date	
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City	Guilford	State	CT	ZIP	06437	Country	USA

☒ Additional inventors are being named on the 1 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

Title: Method for Reducing Artifacts
in Nucleic Acid Amplification
Filed: February 25, 2000

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ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 1 of 1

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor
Given Name (first and middle [if any])	Family Name or Surname
Fawad	Faruqi

Inventor's Signature						**Date**	
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City	Guilford	**State**	CT	**ZIP**	06437	**Country**	USA

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor
Given Name (first and middle [if any])	Family Name or Surname

Inventor's Signature						**Date**	
Residence: City		**State**		**Country**		**Citizenship**	
Post Office Address							
Post Office Address							
City		**State**		**ZIP**		**Country**	

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor
Given Name (first and middle [if any])	Family Name or Surname

Inventor's Signature						**Date**	
Residence: City		**State**		**Country**		**Citizenship**	
Post Office Address							
Post Office Address							
City		**State**		**ZIP**		**Country**	

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